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Pharmacological manipulation of hemoglobin-oxygen affinity in human red blood cells

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PHARMACOLOGICAL MANIPULATION OF HEMOGLOBIN-OXYGEN AFFINITY IN HUMAN RED BLOOD CELLS

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CONTENTS

INTRODUCTION

The successful transition from the intra-uterine environment to the gaseous atmosphere is the first and most important task in the life of the newborn. The problem of oxygenating the tissues is solved in the fetus by direct diffusion across the placental membranes, and transport by fetal hemoglobin. Once the lungs have begun functioning, a major adaptation is required in order to ensure uninterrupted oxygen transport. This is gradually accomplished by the transition from fetal to adult hemoglobin, which is more suited to the task of loading oxygen in the lungs and releasing it to the tissues during extra-uterine life. In both fetus and adult, oxygen is carried to the tissues by a hematological "conveyor belt" which must be responsive to alterations in oxygen supply and demand.

This thesis will review the mechanisms by which red blood cells accomplish the task of delivering sufficient oxygen to supply the needs of the tissues, specific adaptations which occur in response to physiological stresses, and methods of altering oxygen carrying capacity. The clinical significance of the role of 2,3-diphosphoglycerate in hemoglobin-oxygen affinity will be emphasized.

Altered oxygen carrying capacity of the blood may be caused by deficient quantities of hemoglobin, alterations of metabolism within the red blood cell, and any factor

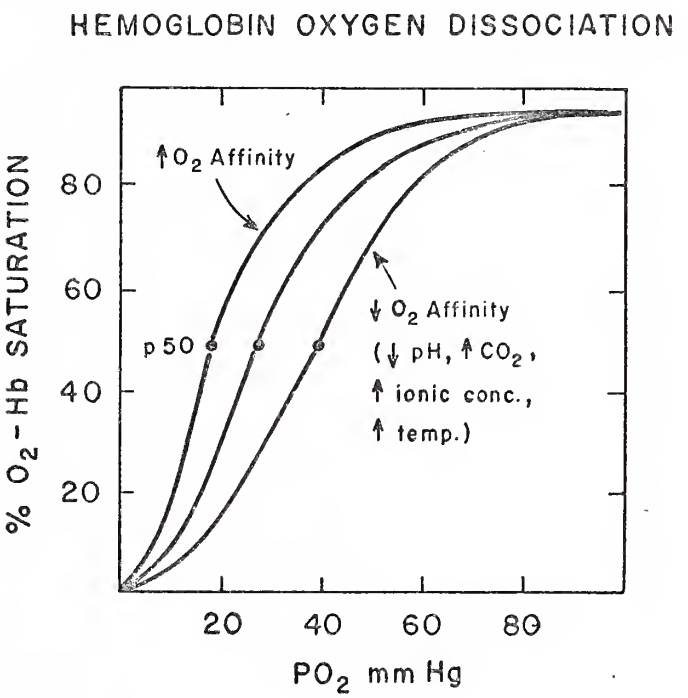
affecting the affinity of hemoglobin for oxygen. Methods of increasing oxygen transport to tissues have primarily consisted of increasing red blood cell mass by means of blood transfusions, and increasing cardiac output. Attempts to alter hemoglobin-oxygen affinity have only recently been investigated. This thesis will present an original laboratory investigation done by the author to determine the effect of corticosteroids on hemoglobin-oxygen affinity.

THE ROLE OF 2,3-DIPHOSPHOSPHOGLYCERATE IN OXYGEN TRANSPORT

The Affinity of Hemoglobin for Oxygen

Tissue oxygenation is dependent upon the oxygen content of inspired air, the partial pressure of oxygen (pO_2), alveolar ventilation, oxygen diffusion into red cells, cardiac output, blood volume, hemoglobin concentration, and oxygen diffusion from the blood into tissues. The affinity of hemoglobin for oxygen is most clearly discussed in terms of the hemoglobin-oxygen dissociation curve (FIG. 1). It can be seen that at partial pressures of oxygen of 110 mm. Hg which exist in the lung, normal adult hemoglobin becomes 95 percent saturated with oxygen. There is very little change in hemoglobin saturation with increases in the pO_2 greater than 110 mm. Hg. When the partial pressure of oxygen is reduced to 27 mm. Hg, hemoglobin is 50 percent saturated. This pO_2 is referred to as the P_{50}. Shifts in the hemoglobin-oxygen dissociation curve to the right correspond to an increase in P_{50}, while shifts of the curve to the left correspond to a decreased P_{50}. Shifts in the curve from normal indicate alterations in the affinity of hemoglobin for oxygen. Factors which decrease the affinity include: decreased blood pH (Bohr effect), increased blood carbon dioxide content, increased ionic concentration, and increased temperature. Increased affinity is found with increased blood pH, and decreased temperature.[68,70]

FIGURE 1


HEMOGLOBIN OXYGEN DISSOCIATION
% O_2-Hb SATURATION
80
60
40
20
↑O_2 Affinity
p 50
↓ O_2 Affinity
(↓ pH, ↑ CO_2,
↑ ionic conc.,
↑ temp.)
20
40
60
80
PO_2 mm Hg

Decreased hemoglobin-oxygen affinity shifts the dissociation curve to the right (increased P_{50}), since a higher pO_2 is required to maintain the same percent saturation. Conversely, increased hemoglobin-oxygen affinity shifts the curve to the left (decreased P_{50}). The physiological significance of decreased hemoglobin-oxygen affinity is that more oxygen can be delivered to tissues without a reduction in partial pressure of oxygen. Thus, despite a decrease in degree of hemoglobin saturation, the end-capillary oxygen gradient is maintained. Bendixen and Laver[6] place the "average critical range" of pO_2 to maintain a diffusion gradient into tissues between 20 and 30 mm. Hg, which corresponds to a hemoglobin saturation of 35-55 percent at normal pH and temperature. It can be seen that a right-shifted curve has a much lower degree of saturation in the "average critical range" of pO_2 than does the normal curve, and thus would be a highly adaptive response to either increased tissue oxygen requirements, or hypoxia.[58] While decreased hemoglobin-oxygen affinity is one mechanism to increase tissue oxygenation, hypoxia and increased oxygen consumption can be compensated for by improved oxygen transport. Increased cardiac output is one way to achieve this, but is limited by increased cardiac oxygen consumption. The other main mechanism for increasing oxygen transport is increased hemoglobin concentration.

Hemoglobin Structure and Function

Each of the two sets of chains present in the hemoglobin A ($alpha_2$ $beta_2$) molecule contains a heme prosthetic group, which is the site of reversable binding with oxygen, primarily by hydrophobic bonds. As each heme group becomes saturated with oxygen, the subsequent heme group is more easily saturated at smaller increments of partial pressure of oxygen. Because of this cooperative "heme-heme interaction," 90-100 percent saturation can occur at partial pressures of 60-100 mm. Hg, and oxygen can be readily released to tissues by small changes in tissue oxygen tension.[76]

The structure of hemoglobin can be contrasted to that of myoglobin, which is a heme containing monomer. There is no facilitation of oxygen saturation at increasing partial pressures of oxygen, and myoglobin does not release oxygen at normal tissue partial pressures.

Investigations by Perutz[64] have shown basic differences in the crystallographic structures of oxygenated and deoxygenated hemoglobin. Studies of nuclear magnetic resonance and electroparamagnetic resonance have shown that direct heme-heme interaction is mostly due to conformational changes in the protein portion of the molecule, mediated through subunits by their common interfaces.[77] Thus, changes in oxygen affinity of hemoglobin due to temperature, pH, ionic concentration, and carbon dioxide result

from interactions between oxygen binding sites and binding sites for other ligands.

The Interaction Between Hemoglobin and Organic Phosphates

In 1967, studies by Benesch and Benesch[8] and Chanutin and Curnish[20] showed that red cell concentrations of organic phosphates had significant effects on hemoglobin-oxygen affinity, independent of the effects of temperature and pH. 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) were shown to bind reversably to hemoglobin A ($alpha_2$ $beta_2$), and in some way decrease hemoglobin-oxygen affinity. Adenosine diphosphate (ADP) also binds to hemoglobin, but does not show as high binding affinity as do 2,3-DPG and ATP.

Benesch[10] showed that adding increasing concentrations of 2,3-DPG to a solution of hemoglobin results in a significant increase in P_{50}. Thus, an important role of 2,3-DPG in red blood cells is to bind to hemoglobin in such a way as to decrease hemoglobin affinity for oxygen (FIG. 2).

Bauer et al.[4] have shown that 2,3-DPG and ATP concentrations were low in stored blood, and this accounted for the increase in hemoglobin-oxygen affinity that occurs with increased age of stored blood.

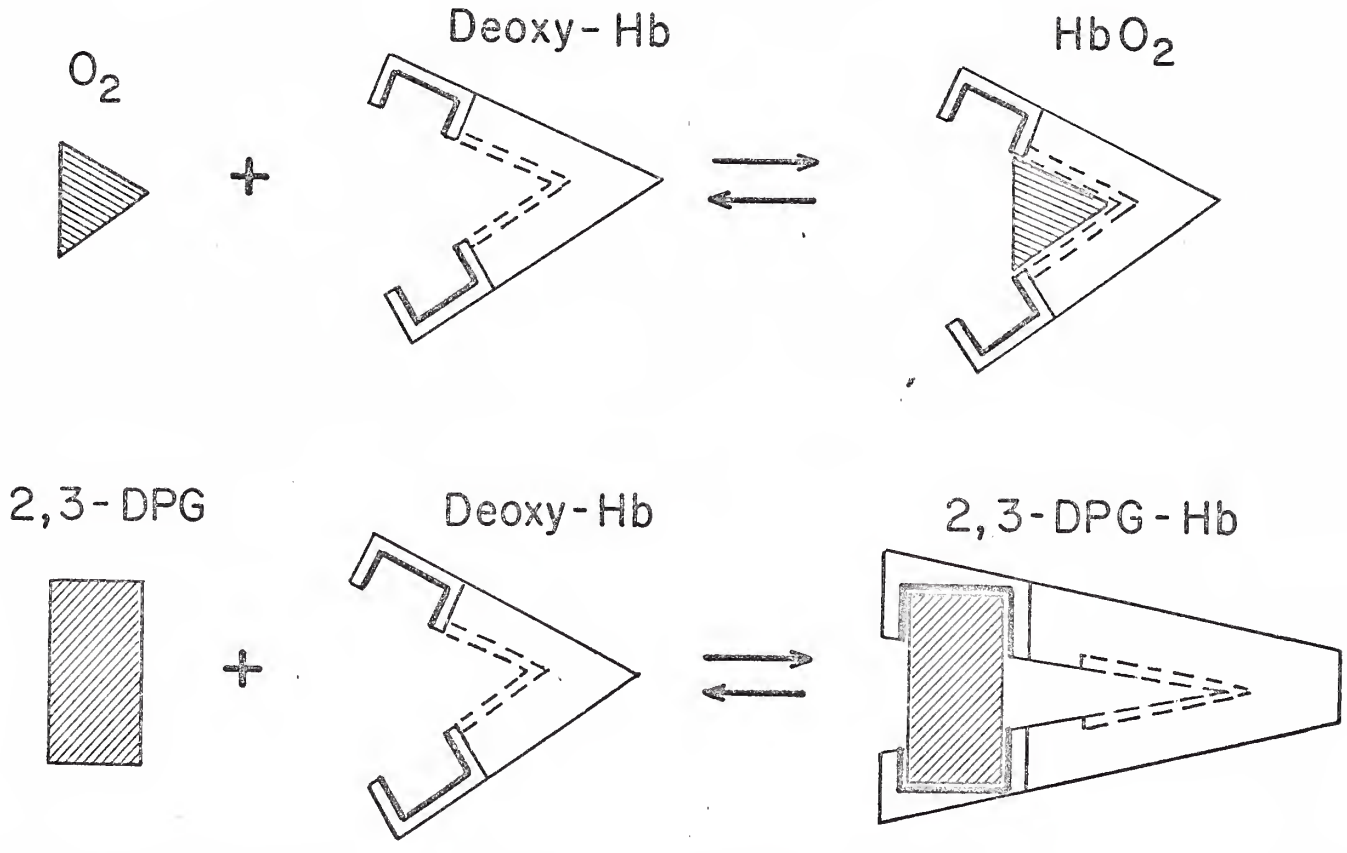


FIGURE 2 A schematic representation of how 2,3-DPG and oxygen can compete for a structural conformation of Hb, but at different binding sites. Binding of 2,3-DPG to deoxy-Hb decreases binding of oxygen to Hb. Binding of oxygen to Hb does not prevent access of 2,3-DPG to its binding site, but reduces affinity of oxy-Hb for 2,3-DPG.

2,3-DPG has a higher binding affinity for deoxygenated hemoglobin than for oxygenated hemoglobin, and does not bind to methemoglobin or cyanomethemoglobin. The significance of this is that 2,3-DPG apparently selectively binds with the deoxy conformation, and stabilizes the molecule such that its affinity for oxygen is reduced.[10] Thus, 2,3-DPG and oxygen effectively compete for the deoxy conformation.

Isolated alpha or beta chains do not bind 2,3-DPG, indicating that binding requires complete tetramers. 2,3-DPG does not bind to alpha-4 tetramers, but does bind to beta-4 tetramers (hemoglobin H) less tightly than to hemoglobin A, implying that the binding site is on the beta chain of hemoglobin.[9]

Beta-4 tetramers maintain a fixed conformation similar to deoxy-hemoglobin, thus reducing heme-heme interaction, the Bohr effect, and increasing oxygen affinity. 2,3-DPG binding to beta-4 tetramers does not reduce oxygen affinity, since beta-4 tetramers are apparently in a fixed conformation which is not affected by 2,3-DPG.[9]

While hemoglobin-2,3-DPG binding can cause shifts in the position of the hemoglobin-oxygen dissociation curve, the sigmoid shape of the curve is retained because of the fact that heme-heme interaction is not altered.[11,15,20]

The exact binding site on the hemoglobin molecule has been described best by Bunn and Briehl[15], and supported by the model of Greer and Perutz[33]. Taking into account preferential bindng to beta chains when the molecule is in the deoxy conformation, as well as the fact that fetal hemoglobin ($alpha_2$ $gamma_2$), without beta chains, shows some reaction with 2,3-DPG, they have proposed that 2,3-DPG is bound at the beta 143 histidine, as well as to the N-terminal amino group of beta and gamma chains. This accounts for the strongest binding to beta chains, some interaction with gamma chains, and no binding to alpha chains. In addition, it appears that the binding sites are common for all the organic phosphates, since competitive interference can be demonstrated between 2,3-DPG and ATP.[32,46]

2,3-DPG Interaction With Different Hemoglobins

Much important work on the oxygen affinity of different hemoglobin molecules was done by Bunn and Briehl[15] in 1970. When 2,3-DPG was added to a solution of normal hemoglobin A, they found oxygen affinity to decrease, as indicated by a doubling of P_{50}. Hemoglobin F ($alpha_2$ $gamma_2$) which has an uncharged serine in the gamma chain replacing the H21 histidine, only had a 20 percent increase in P_{50}.[26] Hemoglobin A_{IC}, a minor electrophoretic component of Hb A, has a hexose residue bound to the N-terminal

groups of the beta chain. This reduces 2,3-DPG binding at that site, and consequently the decrease in oxygen affinity is only 23 percent. Hemoglobin F_1, a minor hemoglobin F component, has an acetyl residue covalently bonded to the N-terminal group of the gamma chain of hemoglobin F, and this molecule does not bind 2,3-DPG either on the alpha chains or the gamma chains, and the oxygen affinity is not altered.[15]

Hemoglobin S ($alpha_2^A$ $beta_2^{glu->val_6}$) solutions show normal oxygen dissociation and interaction with 2,3-DPG. However, in vivo studies of sickle cells have shown a shift in the hemoglobin-oxygen dissociation curve to the right, apparently as a result of intra-cellular changes, and not hemoglobin-2,3-DPG interaction.[67] Rotman et al.[75] have shown oxygen uptake by sickled erythrocytes to be decreased. Charache et al.[18] have shown that 2,3-DPG concentrations in sickle erythrocytes is increased, apparently as a physiological response to the decreased cellular oxygen uptake.

Many abnormal hemoglobins have been found to be associated with alterations in P_{50} without alterations in 2,3-DPG concentrations or binding.[87] However, hemoglobin Hiroshima, which has an amino acid substitution for beta 143 histidine, does show increased affinity for oxygen, presumably because of a decreased alkaline Bohr effect,[65] but also possibly on the basis of interference with

2,3-DPG binding.[26,35,38,48]

Fetal hemoglobin shows an interesting adaptive modification. Because of its diminished interaction with 2,3-DPG, hemoglobin F has a dissociation curve that is shifted to the left of that of hemoglobin A. This allows a greater amount of oxygen to be picked up at the partial pressure of oxygen present in the placenta. Unloading of oxygen is facilitated by the low partial pressure of oxygen in the tissues.[86] Thus, hemoglobin F is uniquely suited to the intra-uterine environment, where oxygen must be picked up by hemoglobin at partial pressures that are much lower than are found in the lung during extra-uterine life.

It would be expected then, that as more and more adult hemoglobin is synthesized, and replaces fetal hemoglobin during the first few months of life the affinity for oxygen would decrease, and the P_{50} would steadily rise. In fact, the P_{50} does increase steadily during the first three months of life,[24] but the decrease in oxygen affinity does not correlate well with the decrease in hemoglobin F concentrations. During the first week of life, 2,3-DPG concentrations rise, although the hemoglobin F concentration does not change. During the second and third weeks of life, the 2,3-DPG concentration returns to the birth level. Thus, while hemoglobin F

concentration is continually falling, and 2,3-DPG concentrations rise and then fall to birth level, P_{50} steadily increases (FIG. 3).

In order to explain this lack of correlation, Delivoria-Papadopoulos[24] created the concept of "the functioning or interacting 2,3-DPG fraction," which takes into account the percent Hb A (2,3-DPG conc. x %Hb A), and shows good correlation with the P_{50}. Even better correlation between 2,3-DPG and P_{50} was demonstrated by Orzalesi and Hay[54a] when the 40% interaction between 2,3-DPG and Hb F is considered in addition to the percent Hb A present ("effective DPG fraction"). Thus, any individual infant's P_{50} is dependent upon his particular Hb A: total hemoglobin ratio, and total 2,3-DPG concentration.

The increase in 2,3-DPG concentration during the first week of life could presumably be a physiological attempt to decrease the high oxygen affinity of hemoglobin F. 2,3-DPG levels decline to normal during the second and third weeks as Hb A concentrations begin to increase, with concomitant decrease in hemoglobin-oxygen affinity.

Premature infants have a hemoglobin-oxygen dissociation curve which is shifted to the left, and which never shifts as far right-ward after birth as does a term infant's. This is presumably due to instability of the 2,3-DPG molecule of an unknown nature. It has been shown that 2,3-DPG mutase is normal in premature infants.[55]

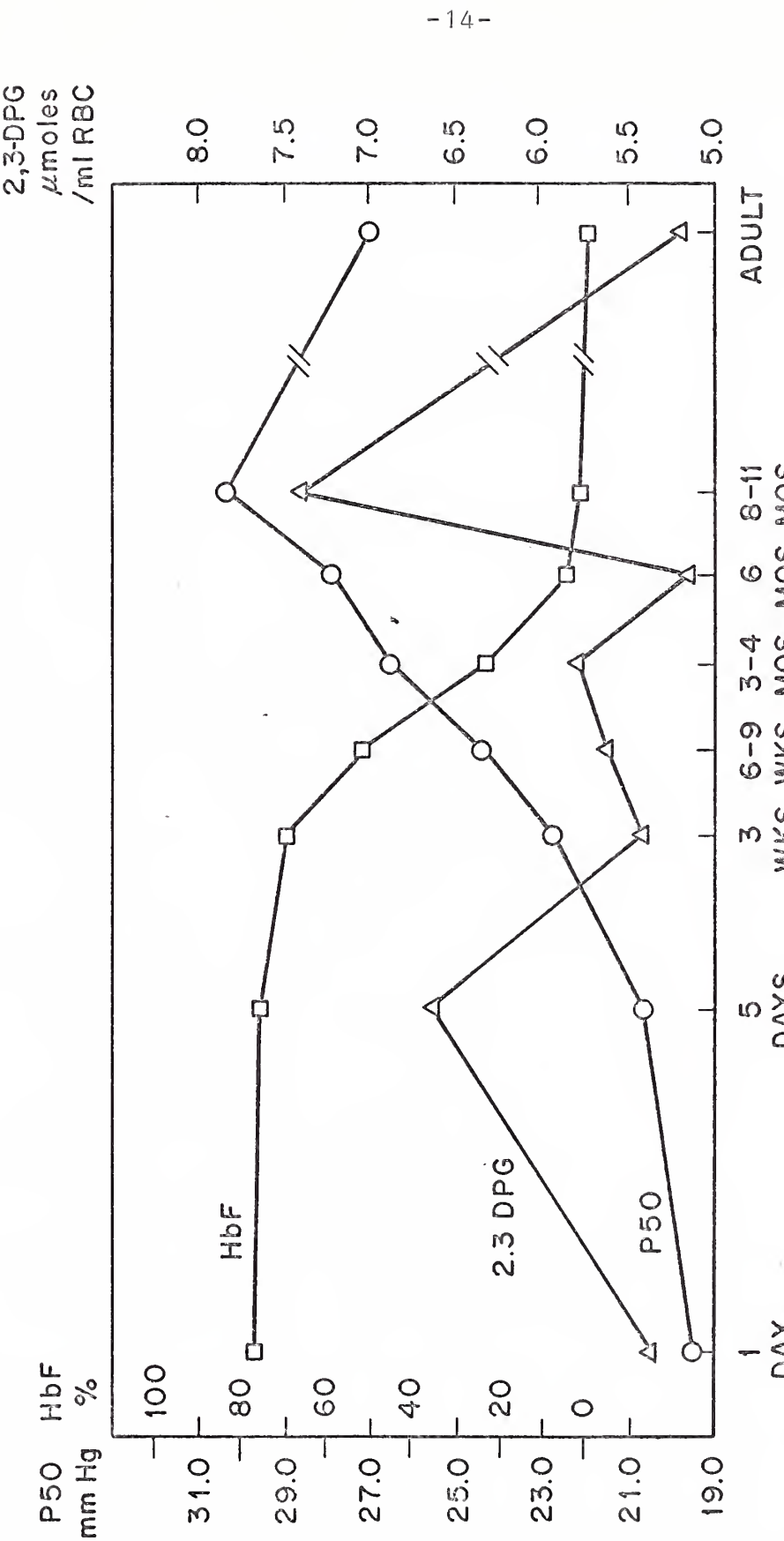


FIGURE 3 RELATIONSHIP BETWEEN Hb F, 2,3-DPG, AND P_{50}

P_{50} rises steadily from birth, while Hb F does not begin to fall until 2-3 weeks after birth, and 2,3-DPG levels rise and then fall during the first 3 weeks of life. The "functioning fraction of 2,3-DPG" correlates well with P_{50}, but does not explain the rise in 2,3-DPG during the first week. (Data from: Oski,FA, and Gottlieb, AJ, The interrelationships between red blood cell metabolites, hemoglobin, and the oxygen-equilibrium curve, Prog. Hema.,8:33-67,1971.)

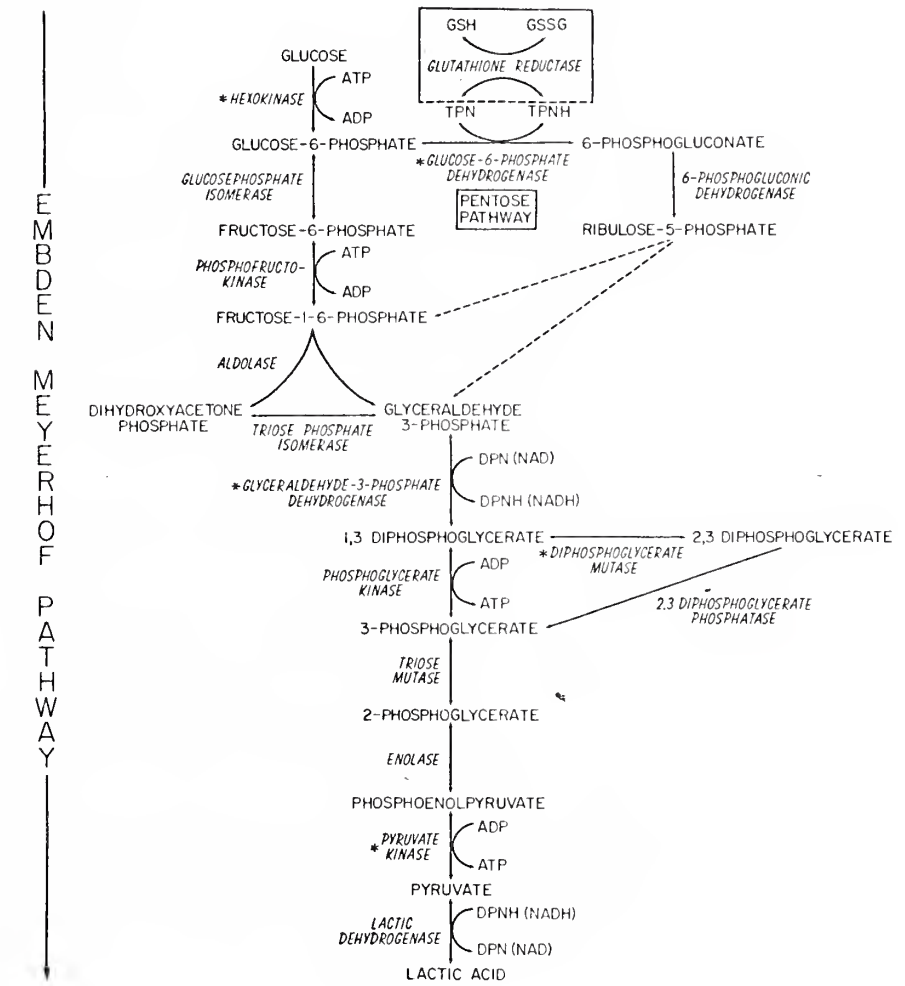
THE BIOSYNTHESIS OF 2,3-DIPHOSPHOGLYCERATE AND ITS REGULATION IN CLINICAL CONDITIONS

Regulation on the Biochemical Level

2,3-DPG synthesis is ultimately dependent on the over-all rate of glycolysis. Primary control is dependent on phosphofructokinase (PFK) activity, which is pH dependent (FIG. 4). ATP concentrations play a regulatory role in that PFK is inhibited by small concentrations of ATP, and this inhibition is overcome by alkalosis and by ADP and inorganic phosphate.[51] Inorganic phosphate and NAD are important in the control of glyceraldehyde-3-phosphate dehydrogenase (G3PD) activity. In the presence of high pH and inorganic phosphate concentrations, red cell glycolysis is increasingly regulated by G3PD activity. In the presence of NAD and inorganic phosphate, phosphoglycerate kinase (PGK) becomes the major regulatory enzyme. PGK activity is dependent on 1,3-DPG concentrations and the ATP:ADP ratio.[30] ADP plays a role in regulating competition between 2,3-DPG mutase and PGK for 1,3-DPG. With higher ADP concentrations, 1,3-DPG is converted to 3-phosphoglycerate (3PG), bypassing 2,3-DPG synthesis. ATP concentrations will decrease PGK activity. 1,3-DPG formation is enhanced by a high pH, high inorganic phosphate concentration, and high NAD concentration.

The synthetic step from 1,3-DPG to 2,3-DPG is

FIGURE 4


EMBDEN MEYERHOF PATHWAY
GSH
GSSG
GLUTATHIONE REDUCTASE
TPN
TPNH
GLUCOSE
ATP
*HEXOKINASE
ADP
GLUCOSE-6-PHOSPHATE
*GLUCOSE-6-PHOSPHATE DEHYDROGENASE
6-PHOSPHOGLUCONATE
PENTOSE PATHWAY
GLUCOSEPHOSPHATE ISOMERASE
6-PHOSPHOGLUCONIC DEHYDROGENASE
FRUCTOSE-6-PHOSPHATE
RIBULOSE-5-PHOSPHATE
PHOSPHOFRUCTO-KINASE
ATP
ADP
FRUCTOSE-1-6-PHOSPHATE
ALDOLASE
DIHYDROXYACETONE PHOSPHATE
TRIOSE PHOSPHATE ISOMERASE
GLYCERALDEHYDE 3-PHOSPHATE
DPN (NAD)
*GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE
DPNH (NADH)
1,3 DIPHOSPHOGLYCERATE
2,3 DIPHOSPHOGLYCERATE
*DIPHOSPHOGLYCERATE MUTASE
PHOSPHOGLYCERATE KINASE
ADP
ATP
2,3 DIPHOSPHOGLYCERATE PHOSPHATASE
3-PHOSPHOGLYCERATE
TRIOSE MUTASE
2-PHOSPHOGLYCERATE
ENOLASE
PHOSPHOENOLPYRUVATE
*PYRUVATE KINASE
ADP
ATP
PYRUVATE
LACTIC DEHYDROGENASE
DPNH (NADH)
DPN (NAD)
LACTIC ACID

catalyzed by DPG mutase, which is strongly inhibited by 2,3-DPG.[73] 2,3-DPG binding to deoxyhemoglobin with consequent release of product inhibition is now felt to be of minimal importance in the stimulation of 2,3-DPG synthesis.[29]

When 2,3-DPG is synthesized in the red cell, synthesis of products distal to 2,3-DPG in the glycolytic pathway may be reduced. If pyruvate concentrations decrease, there is less substrate for lactic dehydrogenase, the NAD reduced by G3PD cannot be reoxidized by lactic dehydrogenase, and consequently glyceraldehyde-3-phosphate and dihydroxyacetone begin to accumulate. In hypoxic conditions under which 2,3-DPG synthesis is stimulated, tissue lactate and pyruvate levels may increase. If pyruvate enters NAD depleted red cells, oxidation of NADH to NAD is facilitated, and conversion of G3P and dihydroxyacetone to 1,3-DPG can occur.[72]

2,3-DPG is degraded by 2,3-DPG phosphatase, which catalyses the reaction:

$$2,3\text{-DPG} + H_2O \longrightarrow 3\text{-PG} + Pi$$

and is activated by chloride and phosphate + chloride, but is far less active than DPG mutase in red cells.[74]

Hypoxia Induced Effects

Lenfant et al.[42] first showed rapid reversable elevations (within 24-48 hours) in 2,3-DPG concentrations

in the red cells of subjects moving from sea level to an altitude of 15,000 feet. As mentioned, acidosis shifts the hemoglobin-oxygen dissociation curve to the right by the Bohr effect, and when subjects are pretreated with an acidosis inducing drug such as Diamox, their oxygen releasing capacity is maintained and the physiologic increase in 2,3-DPG is suppressed during moves to higher altitudes.[43]

Recently, Miller et al.[50] have confirmed Lenfant's findings, and also have proposed that stimulation of erythropoietin by hypoxia, alkalosis, and hypocapnia is suppressed by rising levels of 2,3-DPG and the resulting decrease in hemoglobin-oxygen affinity. They concluded that the rise and fall of serum and urinary erythropoietin levels after exposure to hypoxia are due to changes in hemoglobin-oxygen affinity rather than to bone marrow utilization of the hormone.

In conditions of relatively poor tissue oxygenation red cell 2,3-DPG levels are found to be increased in an adaptive attempt to facilitate oxygen release from hemoglobin. Thus, hypoxic conditions secondary to chronic pulmonary disease,[56] congenital heart disease,[57] chronic liver disease,[56] and heart disease with decreased cardiac output[89] all have associated elevations in 2,3-DPG.

Huehns and Bellingham[36] have shown that in hemolytic anemia, there is an inverse relationship between

packed cell volume and 2,3-DPG level. Valeri and Fortier[83] have shown elevated 2,3-DPG levels in association with decreased red cell mass in the absence of low hemoglobin or hematocrit levels.

Rodriguez and Shahidi[69] found that 2,3-DPG concentrations could be used to differentiate adaptive from pathological deficiencies in red cell volume. In patients with pathological red cell volume and plasma volume deficiencies secondary to panhypopituitarism and to growth hormone deficiency, no increase in 2,3-DPG was noted. However, increases did occur in normal monkeys after growth hormone and thyroxine administration. They concluded that both hormones act synergistically to increase 2,3-DPG concentrations.

The mechanism of 2,3-DPG elevations associated with relative arterial hypoxemia is not entirely elucidated, but much work has been done in this area. Astrup[3] and Duhm and Gerlach[29] have proposed that intracellular pH is the primary controlling factor in 2,3-DPG synthesis, and the release from product inhibition is of minor importance. They propose that with deoxygenation, intraerythrocytic pH rises as a result of hydrogen ion absorption by deoxyhemoglobin. At higher pH levels, PFK is activated, allowing increased glycolysis and 2,3-DPG synthesis. At the same time, increased concentrations of

deoxyhemoglobin are available for 2,3-DPG binding, and any degree of product inhibition present can be overcome.

In patients withsplenomegaly, 2,3-DPG levels are reduced, presumably due to accelerated degradation and impaired red cell glycolysis secondary to erythrocyte sequestration, and the acid environment of the enlarged spleen.[61]

The Effect of Inorganic Phosphate

Lichtman et al.[45] and Travis[82] found decreased red cell glycolysis, decreased 2,3-DPG and ATP levels and increased hemoglobin-oxygen affinity caused by hypophosphatemia. Lichtman[44] also studied uremic patients with hyperphosphatemia and as expected found them to have elevated ATP and 2,3-DPG levels. These results correlate well with the study done by Card and Brain,[19] who found a closer correlation between serum phosphate levels and total organic phosphate level than 2,3-DPG level alone. They studied 62 children, and proposed that the "normally elevated" plasma inorganic phosphate level of children causes elevated red cell organic phosphates, a right shifted dissociation curve, and accounts for the resulting "physiologic anemia of childhood."

Oski and Gottlieb[55] have concluded that, "the red cell 2,3-DPG level in vivo appears to be controlled by

a number of factors which include: the pH, the ratio of deoxy to oxy-hemoglobin, the serum inorganic phosphate concentration, and the level of plasma oxidants."

Alterations in Red Cell Metabolism

2,3-DPG concentrations have been found to be elevated in hyperthyroidism.[49] The mechanism of this increase is not related to hypoxia, but rather either to increased tissue oxygen consumption, or direct stimulation of enzymes involved in 2,3-DPG synthesis. Snyder and Reddy[79] found that triiodothyronine directly stimulates diphosphoglycerate mutase.

Since 2,3-DPG synthesis is a branch of the glycolytic pathway (FIG. 4), metabolic alterations in glycolysis will affect 2,3-DPG synthesis. In subjects with metabolic defects proximal to 2,3-DPG in the Embden-Myerhof pathway such as hexokinase (HK) or glucose isomerase deficiency, low levels of 2,3-DPG and an associated decrease in P_{50} will be found.[57] In subjects with pyruvate kinase (PK) deficiency which would cause a block in the metabolic pathway distal to 2,3-DPG synthesis, high levels of 2,3-DPG are found.[23] Interestingly, these individuals (PK deficient) require only a small increase in cardiac output to tolerate the same amount of exercise that requires doubling of cardiac output in a HK deficient patient.[58]

Nelson and Benson[53] studied 20 Down's syndrome patients, and found 2,3-DPG levels to be significantly higher than in 20 controls. A possible mechanism of this increase is marked increase in the activity of erythrocyte PFK found in Down's syndrome.

Guest and Rapoport[34] in 1948 reported decreased 2,3-DPG levels in comatose diabetics. In 1972, Ditzel[27] studied diabetics recovering from acidosis and found that low 2,3-DPG levels in patients who were acidotic returned toward normal as their acidosis was corrected. His explanation was that in acidosis, 2,3-DPG levels will fall because of deactivation of glycolytic enzymes. At low 2,3-DPG concentrations, the hemoglobin-oxygen affinity will rise, counteracting the acidotic right-ward shift of the Bohr effect. When the acidosis corrected, the hemoglobin-oxygen dissociation curve does not shift right-ward until the 2,3-DPG concentration begins to rise.

In addition, Ditzel[27] has reported that hemoglobin-oxygen dissociation curves in ambulatory diabetics are shifted to the left sufficiently enough to cause 30 percent less oxygen release than in normal controls. In non-acidotic diabetics, Ditzel based the mechanism of low 2,3-DPG levels on chronic hyperglycemia. With excess glucose, sorbitol and fructose concentrations in red cells increase,[52] and the utilization of NADPH in the conversion

of glucose to sorbitol drives the hexose monophosphate shunt. As glycolysis via the Embden-Myerhof pathway declines, 2,3-DPG concentrations fall.

However, three other separate researchers have been unable to reproduce Ditzel's findings in ambulatory diabetics, and reported no significant change in red cell 2,3-DPG concentrations.[1,40,71]

THE PHARMACOLOGICAL MANIPULATION OF 2,3-DIPHOSPHOGLYCERATE

The Use of Inosine in Stored Blood

In 1969, Bunn et al.[16] were able to relate the progressive increase in hemoglobin-oxygen affinity during the first days of storage of blood in acid-citrate-dextrose solutions at 4° C to a decline in red cell levels of 2,3-DPG and ATP. This decline was not observed in blood stored in citrate-phosphate-dextrose solution.[21] The 2,3-DPG decline could also be reduced if inosine is added to the solution, if the pH is maintained at normal levels, and if the hemoglobin is in the deoxygenated state.[37] If the blood is frozen, 2,3-DPG levels can be maintained for long periods.[91]

In patients transfused with ACD stored blood, there is a marked shift in their hemoglobin-oxygen dissociation curve to the left,[85] which reverts back to about 50 percent of normal within 6 to 24 hours after transfusion, as 2,3-DPG is resynthesized.[7,84] In healthy subjects transfusions generally do not cause a clinical problem. However, in critically ill patients massive transfusions (up to 25 units) of ACD blood lowers the P_{50} by 8.6 mm. Hg,[14] and in order to deliver sufficient oxygen to tissues, these patients must compensate by increasing cardiac output. In such cases, Oski et al.[58] has shown the clinical advantage of a right-ward shift of the dissociation curve.

Oski et al.[60] found they could restore 2,3-DPG levels to normal in 2,3-DPG depleted blood by adding inosine, pyruvate, and phosphate. They noted that this effect was related to the purine nucleoside phosphorylase activity of different species. In dogs, which have no PNP-ase activity, there was no rise in the 2,3-DPG level.

In a follow up study, Sugerman et al.[80] observed a 51 percent increase in 2,3-DPG concentration in Rhesus monkey erythrocytes 6 hours after an infusion of inosine, pyruvate and inorganic phosphate. Controls infused with saline had no rise in 2,3-DPG. No change in pH was noted. The mechanism of the rise is the conversion of inosine to hypoxanthine and ribose-1-phosphate (R-1-P). R-1-P can be converted to R-5-P, which enters the hexose monophosphate shunt, and via transketolase and transaldolase can be converted to fructose-6-phosphate and glyceraldehyde-3-phosphate.

The role of pyruvate in the solution is to allow for the oxidation of NADH to NAD, which as previously mentioned, is necessary for the conversion of G-3-P to 1,3-DPG.

The use of inosine in man would require simultaneous administration of a potent uricosuric agent, such as allopurinol, since in the absence of uricase large amounts of uric acid and xanthine, breakdown products of hypoxantine, would accumulate.

Propanolol and Membrane Interaction

Pendleton et al.[63] observed that propanolol shifts the hemoglobin-oxygen dissociation curve to the right. They also noted an accompanying decrease in red cell volume, slight increases in 2,3-DPG concentrations per millilitre of red blood cells, and slight decreases in 2,3-DPG concentrations per gram of hemoglobin. Glucose utilization by red cells was decreased in the presence of propanolol. They concluded that the mechanism of action is not mediated by the beta-blocking adrenergic effect of propanolol since three other beta-blocking agents, INEPA, MJ 1999, and KO 592, did not alter the dissociation curve. Nor is it mediated by direct action on hemoglobin, since there is no effect of propanolol on solutions of hemoglobin. Since alterations in red cell morphology were noted, and since the effect was observed immediately after the addition of propanolol, they proposed that there is an unspecified effect on the red cell membrane.

Oski et al.[59] also found that propanolol does not alter total red cell 2,3-DPG content, does not alter the dissociation curve of pure solutions of hemoglobin or 2,3-DPG depleted blood. They did find that about 30 percent of the 2,3-DPG is bound to the red cell internal membrane, and therefore is unavailable for interaction with hemoglobin. The "bound fraction" apparently serves

as a reserve, which can readily be made available. In propanolol treated red cells, 100 percent of the 2,3-DPG is in the "unbound fraction," which would account for the right shifted curve. They also found that epinephrine prevents the unbinding, implying that the membrane is responsive to vasoactive agents. Since propanolol is a beta adrenergic blocking agent, Oski speculated that there may be adrenergic receptor sites on the red cell membrane. These findings have been disputed by Brann and Newman,[13] who have not been able to find any "membrane-bound" 2,3-DPG in erythrocytes.

A recent abstract by Schrumpf et al.[78] reports significant elevations of P_{50} in patients with angina pectoris and coronary artery disease being treated with propanolol. They speculated that the beneficial effect of propanolol in these patients may be due to increased oxygen delivery to the myocardium as well as to the negative chronotropic effect.

The Effect of Steroids

Means of bypassing the need for transfusions by the use of erythropoietin-stimulating agents were studied by DeGowin et al.[22] They were able to demonstrate increases in hematocrit and plasma erythropoietin levels in one patient with chronic renal failure treated with hemo-

dialysis and testoserone, but no transfusions. No increases were seen in similarly treated anephric patients.[31]

In a larger series, Richardson et al.[66] were able to eliminate the transfusion requirements in 13 of 15 patients with chronic renal failure treated with testosterone for 5-44 weeks.

An in vitro study has shown that 11 ketopregnanolone is an effective inducer of erythroblast multiplication.[12] Another report has found a direct erythropoietic response to testosterone without concomitant rises in erythropoietin levels.[17]

In 1972, Parker et al.[62] reported a significant rise in 2,3-DPG levels in six patients with chronic renal failure treated with testosterone for 12 weeks. They found no alterations in serum inorganic phosphate levels to account for this rise, and suggested only that androgens may have a direct metabolic effect on red cells, or an indirect effect by altering oxygen consumption. P_{50}'s were not studied.

Bauer and Rathschleg-Schaefer[5] had previously demonstrated that in vivo administration of aldosterone and cortisol to rabbits results in elevations of P_{50}, but they did not study 2,3-DPG levels. They concluded that the steroid action was not due to a direct effect

on red cells since their results were not reproducible with in vitro red cell experiments.

Significant elevations in 2,3-DPG levels have been reported by Desai et al.[25] in Rhesus monkeys after a four week administration of testosterone and 11 ketopregnanolone, but no increase after estradiol. They did not observe any change in hemoglobin, red cell mass, or serum inorganic phosphorus levels.

Laboratory Investigation:

PHARMACOLOGICAL MANIPULATION OF 2,3-DIPHOSPHOGLYCERATE LEVELS IN HUMAN ERYTHROCYTES

PURPOSE

The purpose of this study is to determine the effect of corticosteroids on 2,3-diphosphoglycerate levels in human erythrocytes. The relationship of this effect to hemoglobin-oxygen affinity will be evaluated, and other studies on the pharmacological manipulation of 2,3-DPG will be discussed, as well as possible mechanisms of action.

METHODS AND MATERIALS

Two groups of outpatients being treated in the Pediatric Clinic of the Yale-New Haven Hospital were studied during the period from January to September 1973.

Group I

Group I consisted of children being treated with chronic prednisone therapy for nephrotic syndrome. All patients in this group had a diagnosis of lipoid nephrosis (by renal biopsy) or chronic glomerular nephritis. There was no attempt made to disqualify patients from the study on the basis of whether or not they had proteinuria at the time of blood sampling. Patients with any respiratory illness, smokers, or other conditions known to alter hemoglobin-oxygen affinity were not included in the study.

17 patients with a mean age of 8.0 years (8 girls, 9 boys) were being treated with prednisone at dosages from 0.1 mg/kg/day to 2.3 mg/kg/day (mean=0.99 mg/kg/day). The duration of continuous treatment varied from one month to ten months, with a mean of 4.8 months.

20 patients with a mean age of 10.9 (9 girls, 11 boys) with nephrotic syndrome who had not been treated with steroids for at least three weeks prior to the study served as controls.

Laboratory determinations of 2,3-DPG, hematocrit, and pH were done on venous samples of blood in this group.

Group II

10 patients (6 girls, 4 boys) between the ages of four and eight (mean age=5.9 years) with acute lymphoblastic leukemia in remission who were undergoing elective reinduction of remission were studied. These patients were being treated with prednisone (1.5-2.2 mg/kg/day orally) and with vincristine (1.5 mg/m^2/week intravenously) for three weeks, followed by one week of steroid tapering.

Venous blood samples were obtained as follows:

1. Samples taken prior to reinduction, while patients were still on maintenance therapy (methotrexate, cytoxan, and cytosine arabinoside).

2. Samples taken during reinduction with prednisone and vincristine.
3. Samples taken at least two weeks after steroid therapy had been discontinued, and maintenance therapy had been resumed.

In addition, samples were obtained from three patients (1 girl, 2 boys) aged 5, 13, 14 years, who were being treated with vincristine but no prednisone, for at least 3 weeks.

Laboratory determinations of 2,3-DPG concentration, hematocrit, hemoglobin concentration, and serum inorganic phosphate concentration were done in this group.

Blood Sampling Technique

Six cubic centimeters of venous blood were obtained from each patient by simple veni-puncture, and drawn into a plastic syringe. 2 cc of whole blood were heparinized and placed in an ice bath during the transport to the laboratory for 2,3-DPG analysis. 1 cc of heparinized blood was used for hematocrit and hemoglobin determinations.

2,3-DPG Assay

Red cell 2,3-DPG concentrations were determined by the reduced NADP method[41] employed by the Sigma Chemical Company Kit, "Ultraviolet Determination of 2,3-DPG in Blood at 340 mu."

The assay is done on a protein free filtrate prepared from fresh whole blood as follows:

1.0 ml of heparinized whole blood is pipetted into 3.0 ml of cold 8% trichloroacetic acid. The mixture is shaken vigorously, and allowed to precipitate for 5 minutes, and then centrifuged for 10 minutes at 3000 RPM. The clear supernatant is then used for the 2,3-DPG assay, or may be stored at 0-4^{o} C for up to two weeks.

The test solutions are made by mixing in a cuvette the following:

2.5 ml of a solution of 1.0 mg DPNH in 8.0 ml of triethanolamine buffer.

0.1 ml ATP solution (100 mg/ml)

0.25 ml of the protein free filtrate

0.02 ml Glyceraldehyde phosphate dehydrogenase (GAPD) and phospho-glyceric phosphokinase (PGK) suspension in ammonium sulfate.

0.02 ml Phosphoglycerate mutase enzyme suspension in ammonium sulfate, (PGM).

The blank solution is made by mixing in a cuvette exactly the same mixture as in the test solution, except that 0.25 ml of distilled water is used instead of 0.25 ml of the protein free filtrate.

At this time, the optical density (OD)(1st reading) of both the blank and test solutions is read on a Beckman DU-2 spectrophotometer using potassium chromate (30 mg/ 1000 ml) as reference at 340 mu.

0.1 ml of phosphoglycolic acid is then added to each cuvette. The mixture is allowed to stand for 25-30 minutes at room temperature for the reaction to go to completion. Then the OD (2nd reading) is read at 340 mu of the blank and test solutions, using the same reference as before.

Calculations:

1. Δ OD blank = OD of blank (1st reading) - OD of blank (2nd reading)
2. Δ OD test = OD test (1st reading) - OD test (2nd reading)
3. $\Delta OD = (\Delta OD \text{ test}) - (\Delta OD \text{ blank})$
4. umoles 2,3-DPG/ml whole blood $= \frac{(\Delta OD)(3.0)}{(6.22)(0.0625)}$

$= (\Delta OD)(7.7)$

3.0 =volume of solution in cuvette

6.22 =mMolar extinction coefficient for B-DPNH at 340 mu

0.0625=volume of original sample in reaction mixture in ml.

5. nmoles of 2,3-DPG/ml RBC $= \frac{(\text{umoles 2,3-DPG})(100)}{\text{hematocrit}} \times 1000$

Reactions:

1. 2,3-DPG is hydrolyzed to 3-PG by the reaction:

$$\text{2,3-DPG} \xrightarrow{\text{(2,3-DPG Phosphatase)}} \text{3-PG} + \text{Pi}$$

2,3-DPG phosphatase activity is present in solutions of purified phosphoglycerate mutase (PGM). Phosphoglycolic acid acts as a stimulator of this reaction.

2. 3-phosphoglycerate (3-PG) is then converted to 1,3-DPG:

$$\text{3-PG} + \text{ATP} \xrightarrow{\text{(PGK)}} \text{1,3-DPG} + \text{ADP}$$

3. 1,3-DPG is converted to glyceraldehyde-3-P:

$$\text{1,3-DPG} + \text{DPNH} \longrightarrow \text{G-3-P} + \text{Pi} + \text{DPN}$$

When DPNH (NADH) is oxidized to DPN (NAD), there is a resultant decrease in the optical density at 340 mu. Measuring this ΔOD is an indirect measurement of the amount of 2,3-DPG originally present.

pH Determination

pH determinations were done on heparinized blood samples in duplicate by the Radiometer PHM 71 Acid-Base Analyzer.

Hematocrit Determination

Heparinized blood from the same sample used for the 2,3-DPG assay was centrifuged in micro-hematocrit capillary tubes (Fisher Scientific Co.) in a hematocrit centrifuge (International Equipment Co. Model HN) for five minutes. Hematocrits were read on a micro-capillary hematocrit reader (International Eqwipment Co.).

Hemoglobin Determination

0.02 ml heparinized whole blood was added to 5.0 ml Drabkin's solution, mixed and allowed to stand for 10 minutes. Optical density was read on a Beckman DU-2 spectrophotometer at 540 mu, using Drabkin's solution as blank. OD is multiplied by a correction factor of 38.6 to obtain grams of hemoglobin per 100 ml.

Inorganic Phosphate Determination

Serum inorganic phosphates were determined on serum which was frozen immediately after the blood had been drawn by the auto-analyzer of the Chemistry Laboratory of the Yale-New Haven Hospital.

RESULTS

Group I - Chronic Prednisone Therapy

2,3-DPG Levels:

The 2,3-DPG concentration of the treated subjects was 5162±213 nmoles/ml RBC (mean±S.E.M.), which was 14.0 percent higher than the control level of 4527±146 nmoles/ml RBC. When analyzed by Student's t test, this difference was highly significant ($p < .01$). These results are shown in Table 1.

Figure 5 shows the relationship between the dose of prednisone per kilogram body weight and 2,3-DPG concentration. The solid line shows the correlation between dose and 2,3-DPG level of all patients in this group, and was found to be significant ($r = .6113$, $p < .01$). Three patents on steroids had proteinuria at the time of blood sampling, and it can be seen that these patients had higher 2,3-DPG levels at lower steroid dosages (solid circles FIG. 5). The correlation between prednisone dose and 2,3-DPG level is more significant when the three patients with proteinuria are not included with the other 14 patients on steroids who did not have proteinuria ($r = .7860$, $p < .001$)(broken line FIG. 5). 2,3-DPG levels were not significantly different when analyzed by Student's t test between patients with proteinuria and without proteinuria in either the treated group

TABLE 1

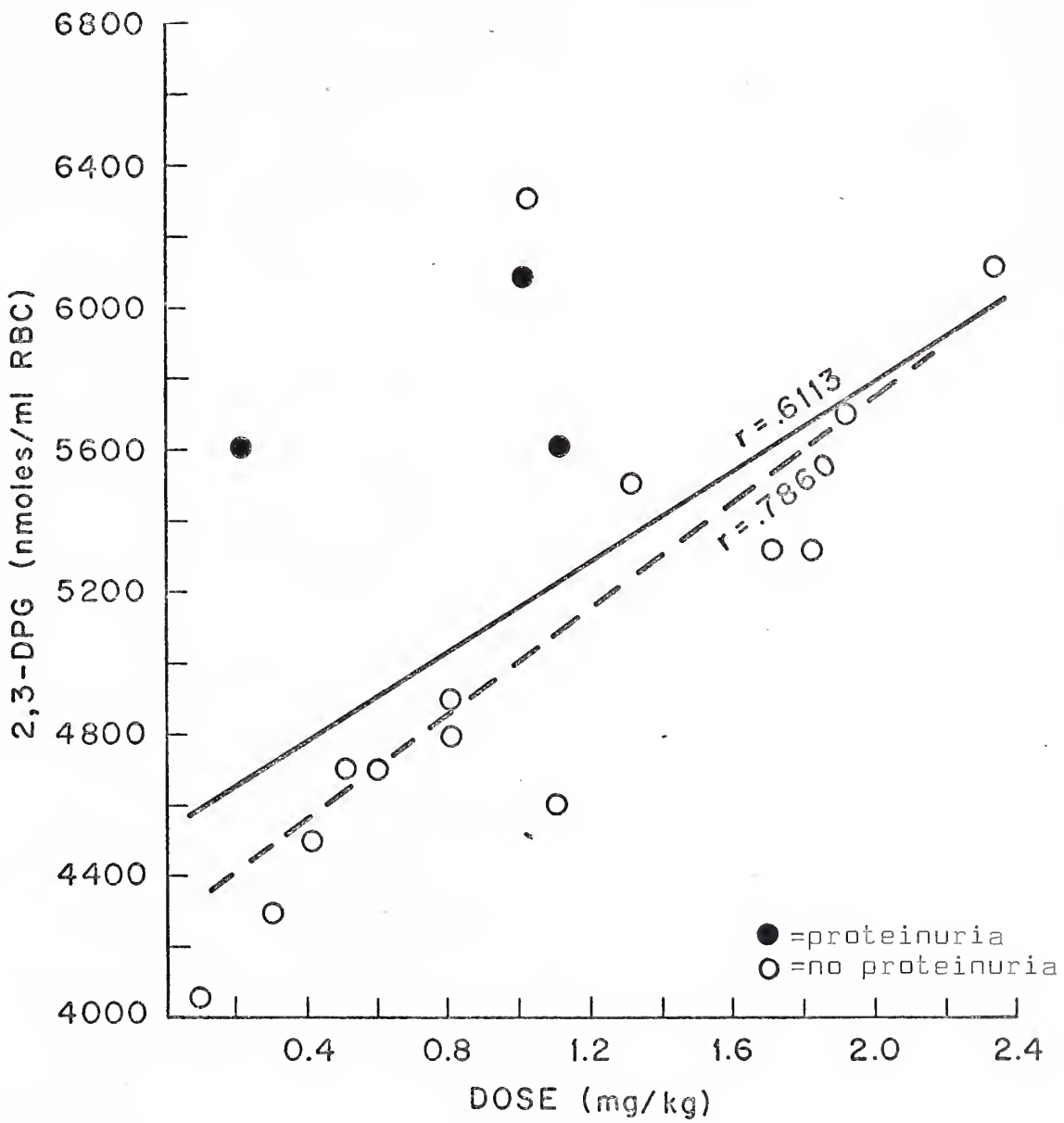
Chronic Prednisone Therapy

	CONTROL	TREATED	p	% INCREASE
2,3-DPG (nmoles/ml RBC)	4527±146*	5162±213	<.01	14.0
Hematocrit (%)	38.6±1.4	44.4±0.8	<.005	15.0
pH	7.38	7.39	N.S.**	

* mean±S.E.M.
**not significant

FIGURE 5

CORRELATION BETWEEN 2,3-DPG LEVEL AND DOSE OF PREDNISONE



(5757±143 (n=3) vs. 5034±596 (n=14)), or the control group (4463±297 (n=8) vs. 4542±218 (n=12)).

There was no significant difference in 2,3-DPG levels between boys and girls in either the treated or the control group.

There was no significant difference in mean pH between the treated (7.39) and the control group (7.38).

Hematocrit Levels:

The hematocrit of the treated group was 44.4±0.8 percent, while the hematocrit of the control group was 38.6±1.4 percent (mean±S.E.M.), an elevation of 14.9 percent which was significant ($p < .005$)(Table 1).

Group II - Acute Prednisone Therapy

2,3-DPG Levels:

The 2,3-DPG concentrations (mean±S.E.M.) before therapy, after 1-2 weeks and 3 weeks of prednisone therapy, after 1 week of steroid tapering, and after 2-3 weeks off therapy are shown in Table 2. The 2,3-DPG concentration of three patients on vincristine alone is also shown in Table 2.

Figure 6 shows the linear rise in 2,3-DPG concentration associated with acute prednisone therapy. After

three weeks of therapy, 2,3-DPG levels rose 21.3 percent, which when analyzed by Student's t test is statistically significant ($p < .02$). Comparison of the rise and subsequent fall in 2,3-DPG levels by Scheffe's method for comparison of two or more groups showed a significant quadratic effect ($p < .01$).

There were no significant differences between 2,3-DPG levels of boys and girls noted.

Hematocrit and Hemoglobin Levels:

Figure 7 shows the linear effect of prednisone therapy on hematocrit and hemoglobin levels. After three weeks of therapy, hematocrit levels were 13.0 percent higher than before therapy, which was statistically significant ($p < .005$).

Hemoglobin levels were 2.0 percent higher after three weeks of therapy, which was not a significant increase by Student's t test. Hematocrit and hemoglobin levels are tabulated in Table 2.

There were no significant differences found between boys' and girls' hemoglobin or hematocrit levels.

Serum Inorganic Phosphate Levels:

Serum inorganic phosphate levels are shown in

TABLE 2

Acute Prednisone Therapy

	BEFORE THERAPY	1-2 WEEKS ON	3 WEEKS ON	1 WEEK TAPER	2-3 WEEKS OFF	VINCRISTINE ONLY
2,3-DPG (nmoles/ml RBC)	5183±316*	6092±439	6286±189	5509±77	4815±199	4879±91
Inorganic Phosphate (mg %)	4.3±0.2	3.6±0.3	4.2±0.4	4.4±0.1	4.8±0.2	3.8±0.3
Hematocrit (%)	37.1±0.6	39.7±1.1	41.9±1.1	40.0±2.9	37.3±1.3	41.3±1.4
Hemoglobin (gm %)	12.8±0.2	13.0±0.2	13.1±0.3	12.9±0.5	12.2±0.4	14.1±0.6

*mean±S.E.M.

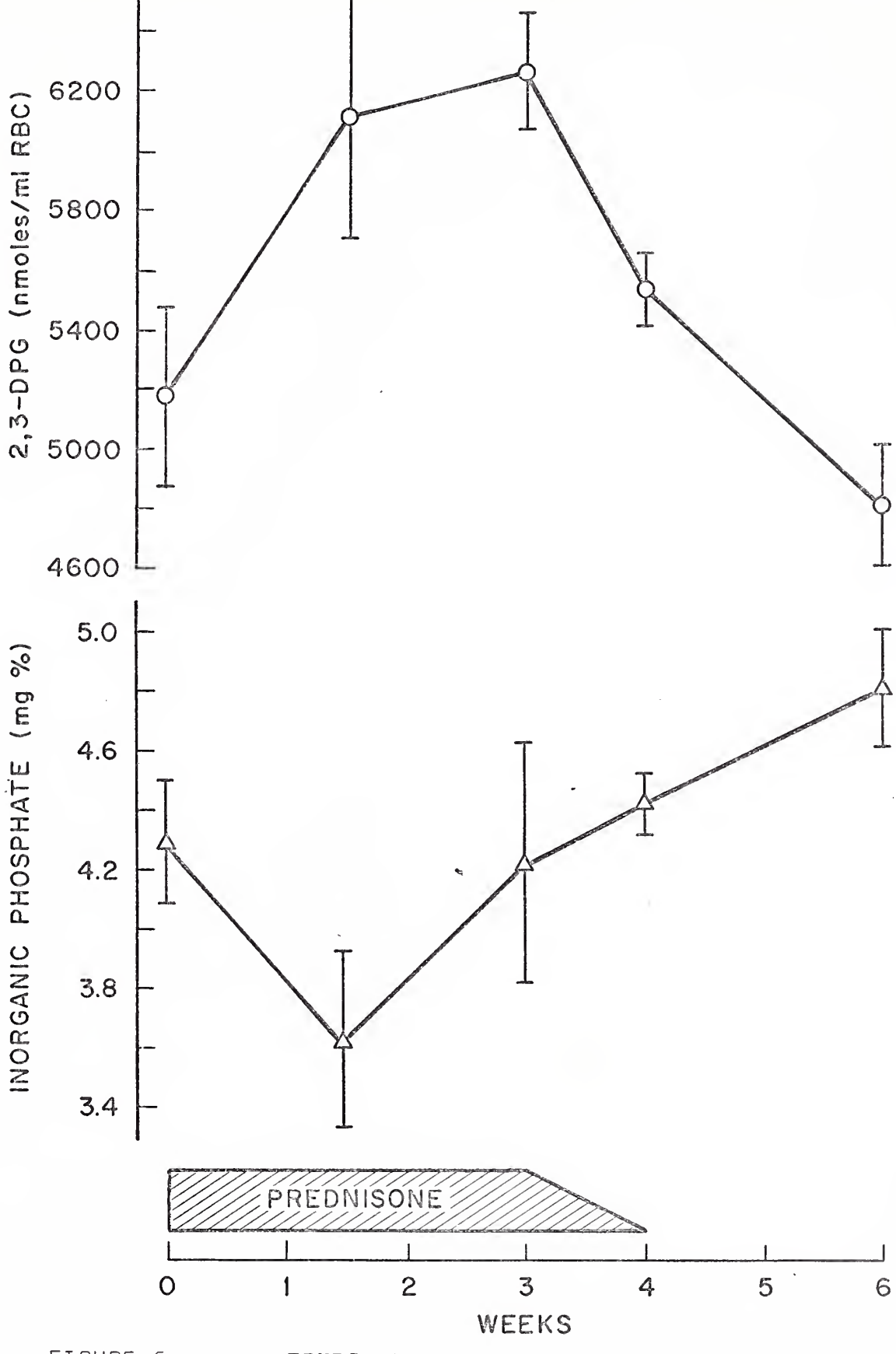


FIGURE 6 TEMPORAL EFFECT OF PREDNISONE ON 2,3-DPG AND INORGANIC PHOSPHATE LEVELS

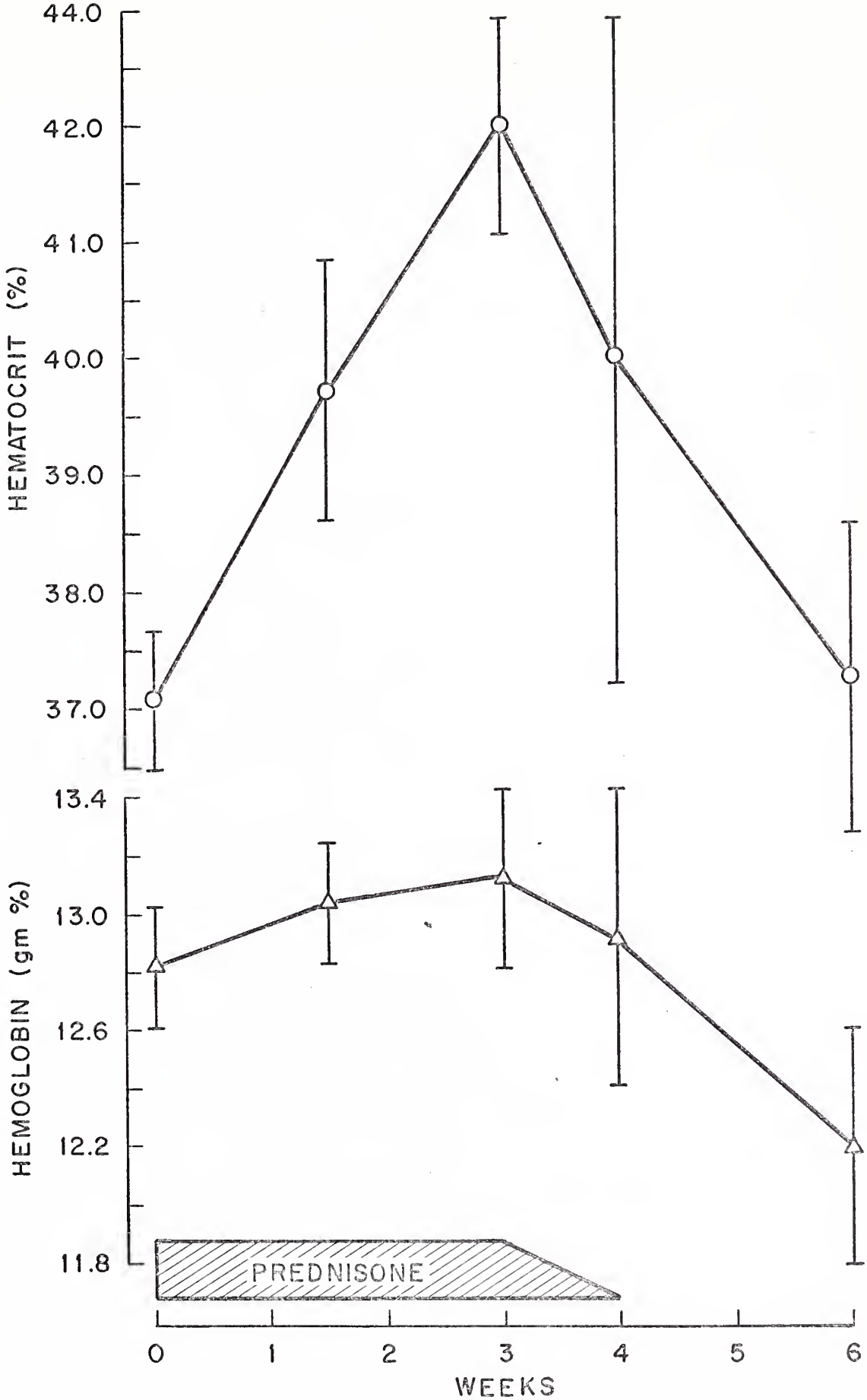


FIGURE 7 TEMPORAL EFFECT OF PREDNISONE ON HEMATOCRIT AND HEMOGLOBIN LEVELS

Figure 6, and tabulated in Table 2. No significant change occurred in inorganic phosphate levels during prednisone therapy, as analyzed by Student's t test. However, there was a decline in inorganic phosphate concentrations during the first two weeks of steroid therapy, which returned to pre-therapy levels by the third week of treatment, and continued to rise after therapy had been discontinued.

DISCUSSION

This study provides the first reported observation of a potent pharmacological manipulation of 2,3-diphosphoglycerate concentrations in human red blood cells that is dose related. In addition, this study provides evidence that prednisone has a significant time related effect on 2,3-DPG levels.

Oski et al.[57] have shown that a 430 nmole/ml RBC increase in 2,3-DPG concentration corresponds to a 1 mm. Hg increase in P_{50}. Therefore, the chronic therapy group would have a hemoglobin-oxygen dissociation curve that is shifted 1.5 mm. to the right, and the acute therapy group would have a curve that is shifted 2.6 mm. to the right.

If the normal dissociation curve is shifted 2 mm. to the right, oxygen release to the tissues would increase by approximately 20 percent, without any decrease

in the normal tissue capillary end-oxygen pressure of 39 mm. Hg. The oxygen extraction required by most tissues is about 5 volumes percent, which is adequately provided at the position of the normal dissociation curve.

It has been shown that in an anemic patient, with a hemoglobin concentration of 7.5 gm/100 ml and an unshifted curve, only about one-half of the oxygen content normally extracted would be released to the tissues. Shifting the curve 4 mm. to the right allows an increased oxygen extraction from 2.5 to 3.5 volumes percent, or an increased oxygen delivery to tissues of 40 percent. Tissue capillary end-oxygen pressure would remain at 39 mm. Hg, well above the critical tension required for adequate oxygen diffusion into tissues.[81]

In this study, we can assume that there was no effect of vincristine on 2,3-DPG levels since the patients treated with vincristine alone did not have significantly different 2,3-DPG levels from the pre-treatment level. The higher hemoglobin and lower serum inorganic phosphate levels of the vincristine group can be accounted for by the relatively older age of these patients.

Determinations of pH, hematocrit, hemoglobin and serum inorganic phosphate levels were done in order to elucidate possible mechanisms of action of prednisone.

Since there was no significant difference between the pH of the control and treated samples in the chronic therapy group, elevated 2,3-DPG levels were not due to alkalosis.

Significant elevations in hematocrit in both groups, and slight increases in hemoglobin confirm studies that show steroid induced erythrocytosis.[12,17] While it has been shown that low hematocrit and hemoglobin levels are associated with high red cell organic phosphate levels,[36] elevated 2,3-DPG concentrations found in this study cannot be attributed to anemia.

Serum inorganic phosphate levels have been shown to have a significant effect on red cell organic phosphate concentrations. Card and Brain[19] found that serum inorganic phosphate levels do not correlate as well with 2,3-DPG levels as they do with red cell ATP levels in conditions characterized by increased serum inorganic phosphate concentration.

As discussed earlier, inorganic phosphate and ADP serve to overcome ATP-induced inhibition of PFK, and inorganic phosphate and NAD regulate G-3-PD activity. In the presence of hypoxia or other stimulation of 2,3-DPG synthesis not due to elevated inorganic phosphate, we would expect increases in 2,3-DPG in preference to ATP, since the 2,3-DPG synthetic pathway bypasses an ATP producing step in the Embden-Myerhof pathway.

This would effectively lower ATP levels and facilitate release of ATP inhibition of PFK. (FIG. 4)

In the absence of hypoxia, 2,3-DPG synthesis is not facilitated by release of product inhibition of 2,3-DPG mutase, or by high pH, and we would expect to find increases in ATP in preference to 2,3-DPG. This would explain the correlation found by Card and Brain, as mentioned above.

In the acute therapy group, decreased serum inorganic phosphate levels were observed during the first two weeks of steroid therapy as 2,3-DPG levels steadily rose. Therefore, we can conclude that prednisone does not act by elevating inorganic phosphate concentrations and thereby stimulate organic phosphate synthesis.

We can speculate that the decline in inorganic phosphate levels is due to increased 2,3-DPG synthesis. A similar temporal relationship between rising 2,3-DPG levels and declining inorganic phosphate levels was noted by Parker et al.[82] during androgen therapy, but they did not speculate on the mechanism of this effect. Young et al.[90] observed a significant decline in serum inorganic phosphate level from 3.68 to 2.41 following initiation of resynthesis of 2,3-DPG in patients with

low 2,3-DPG concentrations secondary to transfusions during cardiac surgery. Their explanation is that bony and other phosphate stores do not maintain plasma phosphate concentrations when exit from the plasma is accelerated by utilization in the synthesis of organic phosphates. They also noted decreased ATP levels in these patients, which they conclude may be due to hypoxia induced 2,3-DPG synthesis and bypassing of the ATP producing step. This mechanism would apply equally well to the observations of the present study.

Further evidence for the validity of this hypothesis is the study by Snyder and Reddy,[79] who have shown that 2,3-DPG synthesis is specifically stimulated by the activation of 2,3-DPG mutase by triiodothyronine, which is also associated with increased ^{32}P uptake by red blood cells.

Two studies have shown inhibitory effects of steroids on glucose-6-phosphate dehydrogenase.[2,54] If this were to occur in red cells, it would make increased G-3-P concentrations available, but would not necessarily account for selective increases in 2,3-DPG. Weber et al.[88] have shown a dose related increase in fructose diphosphatase and glucose-6-phosphatase after hydrocortisone administration.

It is apparent that steroids have potent effects on glycolysis which could stimulate 2,3-DPG synthesis by enzyme induction, but there is little direct evidence for the mechanism by which 2,3-DPG levels specifically may be altered. Increased glycolysis in the tissues could create increased oxygen consumption, and elevations in 2,3-DPG would represent a physiological adaptation to that stress. This mechanism for increased 2,3-DPG levels in this study cannot be ruled out by the data presented.

Steroid interaction with red cell membranes was suggested by Drykorn,[28] who found prednisone to increase survival time of red cells in patients with decreased survival time due to chronic anemia, based on evidence that prednisone stabilizes cellular membranes.

The hematological response to androgens has also been studied in patients with sickle cell anemia. One study reported a clinically beneficial response to androgens in 80 percent of treated patients as measured by a decrease in number of painful sickle cell crises.[39] The mechanism of this effect, as elucidated by electron micrographic studies of sickle cell membranes, is related to sol-gel transformations of the hemoglobin molecule within the cell, and stabilization of the red cell membrane. Significant increases in hemoglobin and

hematocrit were also found in androgen treated patients with sickle cell anemia.[47] However, neither of these studies analyzed hemoglobin-oxygen affinity or 2,3-DPG levels.

It is not clear how steroid-induced red cell membrane stabilization would affect 2,3-DPG function, however in view of recent evidence of interaction between red cell membranes and 2,3-DPG,[59] this mechanism of action must be considered.

CONCLUSION

The significance of the role of 2,3-diphosphoglycerate in oxygen transport has been firmly established. In the past six years, extensive work has shown that many clinical conditions are characterized by alterations in red cell 2,3-diphosphoglycerate concentrations. While at present 2,3-DPG levels are not generally used as a diagnostic test, it is not inconceivable that determinations of 2,3-DPG concentrations in conditions such as chronic lung disease, cyanotic heart disease, and anemia may be valuable in assessing the severity of the disease. Certainly, 2,3-DPG levels are an important index of the oxygen carrying capacity of stored blood.

Pharmacological manipulation of hemoglobin-oxygen affinity by means of restoration of 2,3-DPG in depleted cells has been successful in stored blood. In conditions such as chronic lung disease, cyanotic heart disease, coronary artery disease, and anemia, in which tissue oxygenation may be compromised, increased oxygen release from hemoglobin could be beneficial. While decreased hemoglobin-oxygen affinity could conceivably be helpful in neonatal respiratory distress syndrome, elevations in 2,3-DPG concentration would be of minimal value

because of the poor interaction between 2,3-DPG and hemoglobin F. Although we can look forward to the development of effective therapeutic means for the regulation of hemoglobin-oxygen affinity, further investigation must be done to determine if such regulation would significantly alter the natural history of the disease, or substitute for current therapy.

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